CYTOLOGY OF CUTLERIA AND AGLAOZONIA

A PRELIMINARY PAPER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 129

SHIGÉO YAMANOUCHI

This preliminary note gives a brief account of my cytological studies of *Cutleria multifida* Grev. and *Aglaozonia reptans* Kütz. The material was collected last winter and spring at Naples, where I occupied a table of the Carnegie Institution at the Zoological Station. The work was begun at Naples and was continued at the University of Chicago under the direction of Professor JOHN M. COULTER and Professor CHARLES J. CHAMBERLAIN, to whom I wish to acknowledge my great indebtedness for their suggestions and criticisms. Many points of cytological interest and importance, as well as the discussion of literature, will be presented in the full account to be published later.

Cutleria multifida

GAMETOGENESIS

Cutleria multifida is generally dioecious. The young thallus, 1–3^{mm} long and narrowly fan-shaped, presents no features to distinguish between the male and female plants. When the thallus has reached the stage for the formation of reproductive organs, the habit of the male plant is occasionally different from that of the female; but an extensive comparative study of the forms suggests that there is great variability in habit, so that it seems impossible to distinguish the two sexual individuals by any morphological character except that they bear as a rule exclusively either male or female organs.

VEGETATIVE MITOSIS IN BOTH MALE AND FEMALE INDIVIDUALS.—Both male and female plants, in good condition, always have a hairy growth at the tips of the multifid filaments of the thallus. Any part of the frond in vigorous growth is favorable for the study of vegetative mitosis, but details are more easily followed in the terminal hairs and in the superficial layer of the entire frond.

The cells in these regions are full of plastids, with usually a single nucleus in the center. The nucleus in the resting state is very small, generally about the size of the plastids or sometimes a little smaller. The network is so finely built that it is hard to recognize much chromatin in it. Neither centrosomes nor central bodies with or without radiations seem to be present.

In early prophase the nucleus increases in size until it is twice the diameter of the resting nucleus and occupies a greater part of the cell, pushing aside the numerous plastids toward the periphery. During the growth of the nucleus, there appear just inside of the membrane chromatin knots which are evidently worked out from the chromatin network by the rearrangement of the material. These chromatin knots, which are of course in continuation with less deeply stained chromatin fibrils, are variable in number at first, but gradually there appears a certain number of chromatin knots that are afterward detached from the chromatin fibrils and become chromosomes, 24 in number. The chromosomes after segmentation gradually assume a slightly elongated rod-form and become arranged at the equatorial plate.

A little before the equatorial plate stage, two kinoplasmic accumulations arise from the cytoplasm surrounding the nuclear membrane at two poles. A well-marked central body in the kinoplasmic mass occurs only at late metaphase. The chromosomes split longitudinally and half of each chromosome proceeds to each pole. During this entire process the spindle is intranuclear. At telophase the nuclear membrane disappears and the two sets of daughter chromosomes, in a state of close aggregation, are now surrounded by cytoplasm, and the formation of the nuclear membrane follows.

When the daughter nuclei are organized, the central spindle disappears completely. The cytoplasm lying between the two nuclei begins gradually to assume a coarse, irregular, alveolar structure, and the walls of the alveoli, probably after a change in their material, form a new cell plate.

Thus vegetative mitosis agrees in its essentials in both male and female plants.

Formation of male gametes.—When the male plant is young, the surface of the thallus bears tufts of hairs here and there in somewhat regularly scattered spots. Later, with or without association of hairs, there are produced short filaments which afterward bear male gametangia. Both the filaments and the hairs arise from superficial cells of the thallus.

One of the superficial cells commences to grow more vigorously than the rest and a typical nuclear division takes place. Two or more subsequent divisions result in a short filament of three or more cells, the terminal one of which is destined to be a male gametangium initial, whose nucleus becomes considerably larger than is common in cases of vegetative mitosis. The mitoses which take place up to the formation of the male gametangium initial are typical and the number of chromosomes is 24.

The details of nuclear division are much more easily and distinctly followed in the gametangia. During the prophase of the first division in the gametangium initial, even before the segmentation of chromosomes, the nucleus is marked by two distinct kinoplasmic accumulations at the poles, and their position indicates the axis of the division. The formation of the cell plate between two daughter nuclei is sometimes much more delayed than in cases of vegetative mitosis.

Following the first division in the gametangium there are several cell divisions, the walls being somewhat perpendicular to one another; as a result there is formed the well-known male gametangium of Cutleria, composed of a great number (sometimes as many as 200) of mother cells, regularly arranged in vertical and horizontal tiers. During all of these successive divisions 24 chromosomes appear.

The nucleus, cytoplasm, and plastids in the mother cell undergo a certain peculiar change, and the whole contents of the mother cell enter into the formation of a male gamete. After the maturity of the gamete a tiny hole is developed in the peripheral wall of the mother cell, through which the gamete escapes.

FORMATION OF FEMALE GAMETES.—The formation of tufts of hairs and of filaments bearing female gametangia is similar to that already described for the male plant. The structure of the cells of the superficial layer is apparently like that of the male plant, and so far as the development of hairs and the behavior of nuclei are concerned, there seems to be no distinction; but the difference is remarkable when the superficial cell which is destined to form a female gametangium begins to grow. The growth usually proceeds until the cell becomes two or more times as large as the corresponding cell of the male plant. Curiously enough the nuclear growth does not keep pace with that of the cell; in other words, the nucleus in the superficial cell at the time of division has almost the same dimensions as in the male plant, and this equality persists up to the formation of mother cells.

The first division of the superficial cell is followed by two or more divisions, which result in a short filament whose terminal cell becomes a female gametangium. A number of divisions in the gametangium produces eventually a structure composed of regularly arranged mother cells.

The nucleus and cytoplasm in the mother cell undergo changes similar to those of the male plant, and after a rearrangement of the plastids a female gamete is formed by the transformation of the whole protoplast. The female gamete, thus formed and containing 24 chromosomes, is discharged from the mother cell.

FERTILIZATION AND GERMINATION

As has been stated, the nuclei in both male and female gametes contain 24 chromosomes. When the female gamete loses its motility and becomes quiescent, a free swimming male gamete becomes attached to it and the union of the two protoplasts occurs. For the sake of brevity, the details of the fusion of the two nuclei, following the union of the gametes, will be omitted in this note.

The fusion nucleus in the common mass of male and female cytoplasm rests for a certain length of time. The first segmentation division takes place within twenty-four hours or less after the union of the gametes. So long as the fusion nucleus remains in the resting state, the round contour of the sporeling is still kept, but when the nucleus has begun to show the early prophase, there is noticed at once at a certain part of the sporeling a slight protuberance, and the cell wall of the protuberance is seen to be considerably thickened. The axis of the mitotic figure of the first segmentation is always parallel with the elongated direction.

The number of chromosomes appearing in the prophase is 48. When these chromosomes become arranged in the equatorial plate, the intranuclear figure is well marked between the kinoplasmic masses at the poles. After the organization of daughter nuclei the central spindle disappears, and the formation of a cell plate at the expense of the cytoplasm begins only after the nuclei have grown to a considerable size. During the second and ensuing divisions the same number of the chromosomes is present.

Thus the sporeling from the fertilized gametes of *Cutleria multifida* develops into a structure of the Aglaozonia form of this species, which contains 48 chromosomes.

Aglaozonia reptans

ZOOSPOROGENESIS

The forms which evidently fall under the category of this species show somewhat wide variability in their habit. The mitosis in the vegetative cells of the form was studied. Since the essential features of the division are similar to those of Cutlèria, detailed accounts will be omitted at this time. The fundamental difference between the two forms is that the nucleus of Aglaozonia contains 48 chromosomes, the number persisting up to the formation of the zoosporangium.

Zoosporangia are produced on the upper surface of the thallus. The origin of the structure is as follows: A superficial cell of the thallus slightly elongates and divides, giving rise to two cells, the upper one of which becomes as a rule a zoospore mother cell; not infrequently, however, several cell divisions take place, and in that case the terminal cell becomes the mother cell. The growth of the zoospore mother cell is striking; it elongates until its length becomes three to six times its width. The elongation of the cell is always accompanied by the growth of the nucleus, which remains in the middle region of the cell.

When the nucleus is approaching the prophase, the chromatin network, by a possible rearrangement of the material, becomes less and less branched, and finally there results a tangled mass of continuous threads traversing the nuclear cavity. The tangled threads, becoming more and more uniform in thickness, become transformed into regularly arranged loops centering at a certain part of the cavity, which represents the beginning of the synapsis stage. The synaptic accumulation of the threads occurs always in association with the kinoplasmic mass outside the nucleus, but its relation to the axis of the cell is varied.

The loops shorten and thicken, and finally they break up into 24 bivalent chromosomes, each derived from one of the loops. The heterotypic figure thus established is intranuclear, and the axis of the spindle is in various directions. Between the first and second divisions the daughter nuclei rest. The four nuclei which are products of the second division contain 24 chromosomes, and the same number is found in the third division which gives rise to eight nuclei.

When the zoospore mother cell has reached the eight-nucleate stage, there occurs generally a cleavage of the cytoplasm, which divides the whole contents of the mother cell into eight zoospore primordia (*Anlagen*). Not infrequently, however, one or two more divisions occur after the third, and as a consequence there are produced 16 or 32 nuclei, and in those cases 16 or 32 zoospores are formed.

The nuclear divisions in the mother cell, as well as the segmentation of the zoospore primordia, always occur simultaneously. As was stated before, the chromosomes contained in the thallus of Aglaozonia are reduced to one-half during the first two divisions in the zoospore mother cell, and 24 chromosomes are involved in the zoospores.

The zoospore germinates independently, without any conjugation; possibly 24 chromosomes, the reduced number, may persist in the structure arising from the germination of the sporelings of the zoospores, but the nuclear details in the sporelings have not yet been completely investigated.

Summary

The nuclear conditions during the life-history of *Cutleria multifida* and *Aglaozonia reptans* may be summarized as follows:

1. The nucleus of both male and female plants of *Cutleria multifida* contains 24 chromosomes; and the male and female gametes produced contain the same number.

2. In the union of gametes the number is doubled, and 48 chromosomes appear in the sporelings, which develop into the Aglaozonia form of Cutleria. Therefore it is evident that the individual bearing the name of *Cutleria multifida* represents the gametophytic phase of

the species, 24 being the gametophytic number of chromosomes; and the Aglaozonia form of Cutleria represents the sporophytic phase of the species, 48 being the sporophytic number.

3. *Aglaozonia reptans* contains 48 chromosomes, and the number is reduced in zoospore formation, the zoospore containing 24 chromosomes. The zoospore with the reduced number of chromosomes germinates without conjugation. Although the nuclear details of the sporelings of *Aglaozonia reptans* have not yet been followed, it seems evident that *Aglaozonia reptans* represents the sporophytic phase of the individual whose gametophytic and sporophytic numbers of chromosomes are respectively 24 and 48. Probably *Aglaozonia reptans* as it occurs in nature is identical with the Aglaozonia form of *Cutleria multifida* which we have grown under culture and is now determined to be the sporophytic phase of the species.

THE UNIVERSITY OF CHICAGO